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Quality and safety assessment during the ozonation of cloudy apple juice

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Abstract

Traditionally, ozone processing within the food industry has focused on solid foods by either gaseous treatment or washing with ozonated water. However, with the FDA's approval of ozone as a direct additive to food, the potential for liquid applications has emerged. This study investigates the effect of ozone processing on microbial inactivation (*E. coli* ATCC 25922 and NCTC 12900), and quality parameters (colour, phenolic content) of apple juice. Apple juice samples were ozonated at room temperature ($20 \pm 1.5^\circ\text{C}$) with an ozone concentration of $0.048 \text{ mg O}_3 \text{ min}^{-1} \text{ ml}^{-1}$ and treatment time of 0 to 10 min at a constant flow rate of 0.12 L min^{-1} . *E. coli* inactivation in apple juice was fitted using the Weibull and shoulder log-linear model. Ozone treatment of *E. coli* ATCC 25922 in apple juice resulted in complete inactivation within of 5 min treatment. Apple juice colour (L^* , a^* and b^*) and total phenols were significantly affected by ozone concentration and treatment time. The results presented demonstrate that a desired 5 log reduction can be achieved within 5 min.

Keywords: Apple juice, polyphenol, ozone, *E. coli*

1. Introduction

Apple juice is consumed by people of all ages for its sensory and nutritional qualities. Apple juice is a rich source of antioxidants, and is devoid of sodium, cholesterol, and fat (Lee and others, 2003; Leontowicz and others, 2003). Apples are an excellent source of several phenolic compounds and the presence of polyphenols in apples are recognized for their health promoting antioxidant properties (Lea and Timberlake, 1974; Lea, 1990; Robards and others, 1999; Sanoner and others, 1999; Bushway and others, 2002; Van der

Sluis and others, 2002; Khanizadeh and others, 2008). Polyphenols work as terminators of free radicals and chelators of metal ions that are capable of catalyzing lipid oxidation (Lobo and others, 2009). The presence of soluble free phenolics show the distinct advantage of apple juice consumption when compared to 10 other commonly consumed fruits (Sun and others 2002). The chemical composition of apple juice and cider has been shown to affect the microbial populations within these beverages (Reinders and others, 2001). Because of the acidic pH, apple juice traditionally was not considered as a potential vector for foodborne pathogens. Nevertheless, several foodborne illness outbreaks caused by *E. coli* O157:H7 and *Salmonella* were associated with consumption of unpasteurized apple juice and orange juice (CDC, 1996). The enhanced acid resistance of *E. coli* O157:H7 has led to food borne outbreaks in acidic fruit juices. These outbreaks led the United States Food and Drug administration (FDA) to issue hazard analysis and critical control points (HACCP) regulations for safe and sanitary processing of juice (USFDA, 2001). A primary performance standard is a minimum 5-log reduction of the pathogens of concern in the juice being processed (USFDA, 2001). Thermal pasteurization of fruit juices is the conventional method used for preservation of juices. It has certain adverse effects on product quality, which affects the overall acceptability of the products. Consumers tend to prefer recently extracted juices with fresh taste and minimal flavor or vitamin losses (Bignon, 1997). In order to meet consumer demand, alternatives to thermal pasteurization such as ozone treatment are now being investigated. The FDA's approval of ozone as a direct additive to food in 2001 triggered interest in ozone applications. A number of commercial fruit juice processors in the US

and Europe began employing ozone for pasteurization resulting in the issue of industry guidelines (FDA, 2004).

Ozone is a powerful broad-spectrum antimicrobial agent that is active against bacteria, fungi, viruses, protozoa, and bacterial and fungal spores due to its potential oxidizing capacity (Khadre and others, 2001). Ozone as an oxidant is used in natural water treatment, washing and disinfecting of fruits and vegetables, and juice processing to inactivate pathogenic and spoilage microorganisms (Cullen and others, 2009). In a gas or aqueous phase, ozone has been used to inactivate microorganisms and decontaminate microflora on meat, poultry, eggs, fish, fruits, vegetables and dry foods (Fan and others, 2007). Ozone inactivates microorganisms through oxidization and residual ozone decomposes to nontoxic products (i.e. oxygen) making it an environmentally friendly antimicrobial agent for use in the food industry (Kim and others, 1999).

The objectives of this study were to determine i) the efficacy of gaseous ozone treatment for reduction of *E. coli* at ambient temperature, ii) the effect of prior acid exposure of the challenge microorganism on treatment efficacy and iii) the effect on quality parameters of apple juice.

2. Materials and Methods

2.1 Microorganisms

Two strains of *E. coli* were used in this study: *E. coli* ATCC 25922 (generic strain), obtained from microbiology stock culture of the School of Food Science and Environmental Health of the Dublin Institute of Technology, and *E. coli* NCTC 12900 (non-toxigenic strain), obtained from National Collection of Type Cultures of the Health

Protection Agency (London, UK). Strains were maintained as frozen stocks at -70°C in the form of protective beads, which were plated onto tryptic soy agar (TSA, Scharlau Chemie) and incubated overnight at 37 °C to obtain single colonies before storage at 4 °C.

2.2 Apple juice

Unpasteurized apple juice was purchased from a local processor (Artizan, Dublin 2, Ireland), dispensed in 20 ml sterilins under aseptic conditions and stored at -20°C. The juice purchased was free of added preservatives. For the experimental study, stored apple juice samples were thawed using cold water. The pH of apple juice was measured using a pH meter with a glass electrode (Orion Model, England) and was approximately 3.8.

2.2 Chemicals.

Procyanidins A1, B1, and B2 were purchased from Extrasynthèse (Lyon, France). Caffeic acid, +-catechin, chlorogenic acid, 2,2-Diphenyl-1-picrylhydrazyl (DPPH), epicatechin, ferulic acid, Folin-Ciocalteu Reagent (FCR), gallic acid, 5-(Hydroxymethyl)furfural (HMF), 6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), p-coumaric acid, phloridzin, rutin, syringic acid, trans-4-hydroxy-3-methoxy cinnamic acid, were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO).

2.3 Preparation of cell suspensions

Cells grown in TSB were harvested by centrifugation at 10,000 rpm for 10min at 4 °C. The cell pellet was washed twice with sterile phosphate buffered saline (PBS, Oxoid LTD, UK). The pellet was re-suspended in PBS and the bacterial density was determined by measuring absorbance at 550nm using McFarland standard (BioMérieux, Marcy - l'Etoile, France). The inoculum was then diluted in maximum recovery diluent (MRD,

Scharlau Chemie) to obtain approximately 10^7 CFU mL⁻¹. The cell concentration was further diluted in apple juice to yield a final concentration of 10^6 CFU mL⁻¹.

2.4 Acid exposure of bacterial cultures

Cells were exposed to acid as described by Cheng, Yu and Chou (2003). Acid stress conditions were imposed for two time periods; 1 hour and 18 hours. Working cultures were grown overnight in TSB at 37 °C. Cells were then harvested by centrifugation at 10,000 rpm for 10min at 4°C and the cell pellet was washed twice with sterile PBS, re-suspended in 10 mL TSB (pH 5.0, adjusted with 6N HCl) and incubated at 37 °C for 1h. For the 18-h acid exposure, bacterial strains were grown directly in TSB (pH 5.0) at 37°C. After incubation, cultures were diluted in MRD (pH 5.0) to yield approximately 10^7 cells/mL, with further dilution in apple juice to a final concentration of 10^6 CFU/mL.

2.5 Ozone treatment

Ozone gas was generated using an ozone generator (Model OL80, Ozone services, Burton, Canada). Ozone was produced by a corona discharge generator. Pure oxygen was supplied via an oxygen cylinder (Air Products Ltd., Dublin, Ireland) and the flow rate was controlled using an oxygen flow regulator. The ozone treatment was carried out at mild conditions with a flow rate of 0.12L min⁻¹ and ozone concentration of 0.048 mg O₃ min⁻¹ mL⁻¹. Ozone concentration was recorded using an ozone analyzer. Excess ozone was destroyed by an ozone destructor. To prevent excess foaming, 5-10 µL sterile anti-foaming agent (Sigma Aldrich, Ireland Ltd.) was added before each ozone treatment. Two bacterial strains (*E. coli* ATCC 25922, *E. coli* NCTC 12900) were individually

investigated for their response to ozone treatment. Experiments were performed with non-acid exposed control cultures as well as a range of acid exposed cultures, i.e., 1h and 18h acid exposure, and then inoculated in the apple juice. Apple juice samples were then treated for 6-7 minutes with frequent sampling intervals. All experiments were carried out in duplicate and replicated at least twice.

2.6 Microbiological analysis and modeling the microbial inactivation kinetics

The efficacy of treatments was determined in terms of reduction in viable counts over time. Samples (1mL aliquots) were withdrawn from the treated juice at specific time intervals, serially diluted in MRD and 0.1mL aliquots of appropriate dilutions were surface plated onto the respective media. 1mL of undiluted sample was spread onto 3 plates in order to achieve a detection limit of 1 log CFU mL⁻¹ according to EN ISO 11290-2 method. Populations of challenge organism were determined by the direct surface plating method and the overlay method. In the direct surface plating method, 100 µL of dilution or 1mL of treated sample was surface plated on sorbitol MacConkey's agar plates. Plates were incubated at 37 °C for 24h. In the overlay-plating method, treated sample was surface-plated to previously poured TSA plates, followed by incubation at 37 °C for 2h. The plates were then overlayed with SMAC, incubated at 37 °C for 22h and then counted. Results were reported as Log₁₀CFU mL⁻¹. Data were pooled and average values and standard deviations determined. Means were compared using ANOVA followed by LSD testing at $p < 0.05$ level (SPSS, version 15.0).

The inactivation kinetics of *E. coli* in apple juice showed non-linearity. The non-linear behavior was explained by a Weibull model and a shoulder-log linear model. The

GInaFiT tool was employed to perform the regression analysis of the microbial inactivation data (Geeraerd, Valdramidis & Van Impe, 2005). The format of the Weibull model that was used to analyze the data is as follows:

$$\log_{10}(N) = \log_{10}(N_0) - \left(\frac{t}{\delta}\right)^p \quad (2)$$

where N is the number of microorganisms, N_0 (CFU mL⁻¹) is the initial number of microorganisms, δ (min) (time for the first decimal reduction) and p [-] are parameters related to the scale and shape of the inactivation curve, respectively (Mafart *and others*, 2002).

The numerical values of δ and p were used to calculate a desired log reduction. The time required to obtain a x log reduction (t_{xd}) was calculated using equation 2. For this case study x was equal to 5.

$$t_{5d} = \delta \times (x)^{\frac{1}{p}} \quad (3)$$

The other model used to describe non-linearity was the shoulder-log linear model (Geeraerd *and others*, 2000):

$$\log_{10}(N) = \log_{10}(N_0) - \frac{k_{\max} \cdot (t)}{\ln(10)} + \frac{\log_{10} e^{(k_{\max} \cdot S_l)}}{(1 + e^{(k_{\max} \cdot S_l)} - 1)} \cdot e^{(-k_{\max} \cdot t)} \quad (4)$$

where N is the number of microorganisms, N_0 (CFU mL⁻¹) is the initial number of microorganisms, S_l is the shoulder length. The numerical values of S_l and k_{\max} were used to calculate a desired log reduction. The time required to obtain x log reduction (t_{xd}) was calculated using equation 4. For this case study x was equal to 5.

$$t_{xd} = S_l + (x) \cdot \frac{\ln(10)}{k_{\max}} \quad (5)$$

2.7. Color measurement

Color of ozonated juice samples was measured using a HunterLab colorimeter (ColorFlex, ModelA60-1010-615, Hunter Associates Laboratory Inc., Reston, Virginia, USA). The instrument (65°/0° geometry, D25 optical sensor, 10° observer) was calibrated using white ($L = 92.8$; $a = -0.8$, $b = 0.1$) and black reference tiles. The color values were expressed as L^* (whiteness or brightness/darkness), a^* (redness/greenness) and b^* (yellowness/ blueness) total color difference (TCD) (Equation 1) which indicates the magnitude of color change after treatment were recorded. Color measurements were taken in triplicate from each sample with the mean values reported

$$TCD = \sqrt{(L^* - L_o)^2 + (a^* - a_o)^2 + (b^* - b_o)^2} \quad (1)$$

where, L_o , a_o and b_o are color values of untreated juice

2.8. Polyphenol analysis

2.8.1. FCR assay

Total phenolic content of apple juice was assessed using a modified version of the Folin–Ciocalteu assay (Singelton, Orthofer, & Lamuela-Raventos, 1999). Gallic acid was used

as a standard and the aqueous gallic acid solution (200 mg L^{-1}) was diluted with distilled water to give appropriate concentrations for a standard curve. For the analysis, $100 \text{ }\mu\text{L}$ of methanolic fruit extract or gallic acid standard, $100 \text{ }\mu\text{L}$ of methanol, $100 \text{ }\mu\text{L}$ of Folin–Ciocalteu reagent and $700 \text{ }\mu\text{L}$ of Na_2CO_3 were added into 1.5 ml micro-centrifuge tube. The samples were vortexed immediately and the tubes were incubated in the dark for 20 min at room temperature. After incubation all samples were centrifuged at $13,000 \text{ rpm}$ for 3 min . The absorbance of the supernatant was then measured at 735 nm in 1 ml plastic cuvettes using a spectrophotometer (UV-1700 Pharma Spec, Shimadzu, Japan). The results were expressed in $\text{mg gallic acid equivalent/100 mL}$ ($\text{mg GAE } 100 \text{ mL}^{-1} \text{ juice}$).

2.8.2. Polyphenolic profile (HPLC–DAD)

HPLC-analysis was performed on a Shimadzu SCL 10A chromatography system (Shimadzu, Kyoto, Japan), equipped with a LC-10 AT VP pump, a CTO-10 AC column thermostat, a SIL 10 AD autosampler and a SPD-M10A diode array detector with an absorbance detection range between 190 to 800 nm . Separations were conducted on a Zorbax SB C18, $5\mu\text{m}$, $150 \times 4.6\text{mm}$ column (Agilent Technologies, Santa Clara, CA). The gradient profile was based on a method of Tsao and Yang (2003). Acetic acid in 2 mM sodium acetate (final $\text{pH } 2.55$, v/v) was used as eluent A and 100% acetonitrile was used as eluent B. The column temperature was set at $37 \text{ }^\circ\text{C}$ and the flow rate was 1 mL/min . The solvent gradient program was set as follows: initial conditions 95% A, 5% B; $0\text{--}45 \text{ min}$, $5\text{--}15\%$ B; $45\text{--}60 \text{ min}$, $15\text{--}30\%$ B; $60\text{--}65 \text{ min}$, $30\text{--}50\%$ B; $65\text{--}70 \text{ min}$, $50\text{--}100\%$ B. Prior to injection sample extracts were filtered through PVDF syringe $0.22 \text{ }\mu\text{m}$ filters. The injection volume was $10\mu\text{L}$. Hydroxybenzoic acids, dihydrochalcones,

flavanones and flavanols were monitored at a wavelength of 280 nm, hydroxycinnamic acids at 320 nm and flavonols at 360 nm. For quantification and identification purposes standard curves of analytes of interest were prepared using methanolic solutions of the standards listed above. For quantification and identification purposes external calibration curves were prepared of the standards listed above. The levels of all cinnamic acids were expressed in μg chlorogenic acid/mL apple juice

3.0 Results and discussions

3.1. Microbial inactivation

Populations of indigenous background microflora in unpasteurized apple juice were generally in the range of 4.3-4.8 log CFU mL⁻¹.

Initial populations of *E. coli* inoculated in apple juice were between 6.2-6.7 log CFU mL⁻¹. The inactivation of *E. coli* in apple juice was fitted using the Weibull and the shoulder log-linear model, which provided estimations of microbial inactivation in terms of processing time required. The low RMSE values (Table 1) showed that both models gave a good fit for the experimental data analysed. The estimated t_{5d} values are also shown in Table 1. Both strains of *E. coli* studied (*E. coli* ATCC 25922, *E. coli* NCTC 12900) were sensitive to ozone. Ozone treatment of non acid exposed ATCC 25922 in apple juice resulted in complete inactivation in 5 min of treatment time as determined on TSA-SMAC by overlay method and SMAC, respectively (Fig 1). However, non acid exposed *E. coli* NCTC 12900 decreased by 4.52 and 4.54 log cycles in 5 min of treatment time as

determined on TSA-SMAC and SMAC respectively (Fig 1). The t_{5d} value for NCTC 12900 was higher when compared with ATCC 25922.

3.2. Ozone inactivation of acid exposed *E. coli* cells

Ozone inactivation curves for acid exposed *E. coli* cells at the different acid exposure conditions are shown in Figure 2, 3. The t_{5d} values for 18h acid exposed cells of ATCC 25922 were higher by comparison with both the 1h acid exposed and non acid exposed populations (Table 1), indicating a susceptibility to the treatment with a short period of acid exposure. However, the t_{5d} values for NCTC 12900 non acid exposed populations were higher by comparison with the 1h and 18h acid exposed cells (Table 1), signifying susceptibility to the treatment with acid exposure. Ozone treatment of 1h acid exposed *E. coli* ATCC 25922 resulted in a reduction of 6.3 and 6.0 log cycles after 5 min treatment time as determined on TSA-SMAC and SMAC, respectively. However, population of 1h acid exposed *E. coli* NCTC 12900 treated with ozone decreased by 5.9 and 6.0 log cycles after 5 min treatment as determined on TSA-SMAC and SMAC, respectively. On the other hand, with the 18h acid exposed cells, populations of *E. coli* ATCC 25922 and *E. coli* NCTC 12900 were reduced by 5.0 and 5.6 log cycles respectively within 5 min as determined by using TSA-SMAC overlay. Similar trends were observed using SMAC where 18 h acid exposed *E. coli* ATCC 25922 and *E. coli* NCTC 12900 were decreased by 4.99 and 5.3 log cycles, respectively.

The estimated time for a 5 log reduction of non acid exposed *E. coli* NCTC 12900, the non-toxigenic *E. coli* O157:H7 strain, determined by both the models was higher when compared to that for the generic strain 25922. However, when *E. coli* ATCC 25922 were

subjected to a 1h and 18h acid exposure, the estimated time required for a 5 log cycle reduction was increased as determined by both models. There was a significant difference observed for *E. coli* ATCC 25922 between 18h acid exposed population compared to the non acid exposed population ($p>0.05$); whereas there was no significant difference observed between non acid exposed population of *E. coli* NCTC 12900 and those exposed to acid conditions for 1h or 18 h.

The direct application of ozone was found to be effective for the inactivation or reduction of *E. coli* in apple juice. In the present study, t_{5d} values calculated showed that the inactivation of *E. coli* populations by 5 log cycles was achieved in less than 6 min treatment time.

When microorganisms are stressed, an adaptive response may follow which can increase the organisms' tolerance to the same or to a different type of stress (Yousef & Courtney, 2003). Our results showed that the extent of increased acid resistance varied with the strain and acid exposure conditions. Applying acid exposure for longer time (18 h) actually increased the ozone treatment time required for a 5 log reduction for *E. coli* strain 25922 by comparison with the non acid exposed and 1 h acid exposed population.

Leistner (2000), reported that simultaneous exposure of bacteria to different stress factors requires increased energy consumption and leads bacteria to cellular death through metabolic exhaustion. However, in the case of *E. coli* strain 12900, applying a prior acid exposure did not significantly effect the ozone treatment time required by comparison with the non acid exposed.

3.3. Apple juice colour

Hunter colour values for untreated apple juice were 28.22 ± 2.77 , 21.33 ± 0.22 , 32.85 ± 3.13 for L^* , a^* and b^* respectively. During ozonation juice samples were observed to be lighter in colour i.e. increased L^* and b^* value, whereas a^* values of apple juice samples were found to decrease with increase in treatment time and ozone concentration. Mean L^* & b^* value significantly increased ($P < 0.05$) from 28.22 to 41.56 and from 32.85 to 43.84, while mean a^* value decreased from 21.33 to 15.57 (Figure 1). A linear increase in L^* , b^* and TCD was observed with high regression coefficient (R^2) of 0.97, 0.96 and 0.98 respectively. Whereas, a^* value decreased exponentially with $R^2=0.89$ (Table 2). Color degradation of fruit juices due to ozone processing has been reported for orange (Tiwari and others, 2008), strawberry (Tiwari and others, 2009a) and tomato juice (Tiwari and others, 2009b). Various phenolic compounds in apple juice can contribute to colour, e.g. flavonols, phloridzin, and hydroxycinnamic acids (Sanoner and others, 1999). The strong oxidizing potential of ozone is derived from the nascent oxygen atom. It has been reported that ozonation of organic dyes leads to color loss as a result of oxidative cleavage of chromophores (Nebel, 1975) due to the breakdown of conjugated double bonds. Similarly the chromophore with conjugated double bonds of carotenoid pigments may be degraded as above. The ozone and hydroxyl radicals (OH^\cdot) generated in the aqueous solution may open these aromatic rings and lead to partial oxidation of products such as organic acids, aldehydes, and ketones.

3.4. Phenolic content

Phenolic contents of fresh cloudy apple juice was found to decrease significantly from 638.81 (fresh) to 267.95 mg GAE/100 mL at ozone concentration of $40\mu\text{g mL}^{-1}$ for 10

min. Levels of phenols for fresh cloudy apple juice were in the range of those reported by other authors (Mangas and others, 1999; Décordé and others, 2008; Khanizadeh and others, 2008; Lobo and others, 2009). Polyphenolic profile of fresh and ozonated apple juice is shown in Table 2. One of the three major compounds couldn't be identified as such, but it was clear by the spectrum and the retention time that it belongs to the group of cinnamic acids. Therefore, chlorogenic acid, which was the main cinnamic acid present, was used to quantify all levels of cinnamic acids. Three major phenolic compounds namely Chlorogenic acid (234.26 ug/ml), caffeic acid (234.26 ug/ml) and cinnamic acid (234.26 ug/ml) were detected in fresh apple juice. The distribution of many of the phenolics in apple juice is influenced by various factors including genetic or cultivar, extraction method, processing, and enzyme treatment (Spanos and Wrolstad, 1992; Khanizadeh and others, 2008; Khanizadeh and others, 2007).

During ozonation significant reduction in polyphenols were observed with increase in ozonation time however at 4 min of processing 66.52, 73.50 and 65.02% reduction in chlorogenic acid, caffeic acid and the cinnamic acid was observed (Table 3). This reduction could be mainly due to the oxidation of polyphenols due to strong oxidation potential (+2.07 eV) of ozone. The degradation of polyphenols during ozonation may be due to variety of possible chemical reactions. These reactions may be direct reactions of ozone with the target compound or its intermediates and radical reactions between hydroxyl radicals (produced through ozone decomposition catalysed mainly by the hydroxide ion (OH^-)) (Cullen and others, 2009).

4. Conclusion

The efficacy of ozone treatment was found to be a function of juice type, pH and strain of *E. coli* used. Further, the mechanism of the reaction of ozone with organic materials will contribute to establishing the impact of specific radical species on target microorganisms. Significant changes in colour values (L^* , a^* , b^*), total phenol and polyphenolic content of apple juice were observed. Thus, the effects of ozonation on the nutritional properties of liquid foods such as fruit juice or juice products should be considered by processors prior to its adoption as a preservation technique.

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Table 1: Calculation of the time to achieve 5 log reduction based on Equations (3), (5).

RMSE represents the root mean squared error for the regression performed on the inactivation data by using equations (2), (4).

Strain	Condition	t_{5d} (sec.) (Weibull model, Eq. (2))	RMSE	t_{5d} (sec) (Shoulder log- linear model, Eq. (4))	RMSE
<i>E. coli</i>	non acid exposed	$3.96^a \pm 0.48$	0.31	4.00 ± 0.09	0.34
ATCC	1h acid exposure	$4.62^b \pm 0.95$	0.52	4.44 ± 0.25	0.59
25922	18 h acid exposure	$4.98^b \pm 0.89$	0.51	5.00 ± 0.33	0.41
<i>E. coli</i>	non acid exposed	$5.08^a \pm 0.33$	0.31	5.17 ± 0.18	0.22
NCTC	1h acid exposure	$4.91^a \pm 1.05$	0.31	4.58 ± 0.12	0.34
12900	18 h acid exposure	$4.55^a \pm 0.12$	0.20	4.56 ± 0.07	0.21

Table 2. Regression equation and corresponding goodness of fit

Colour parameters	Regression equation	R ²	SE
<i>L</i> *	$y = 1.4124x + 27.563$	0.97	1.09
<i>a</i> *	$y = 21.242e^{-0.0311x}$	0.89	0.88
<i>b</i> *	$y = 1.1638x + 33.027$	0.96	0.94
TCD	$y = 1.8829x + 0.1488$	0.98	1.09

Table 3. Changes in polyphenolic profile (ug/ml) and total phenols in apple juice

Sample	Chlorogenic acid	Caffeic acid	Cinnamic acid	Total phenol
Fresh cloudy juice	234.26±55.85	19.15±2.48	134.37±23.54	638.81±123.27
4 min (0.048 mg O ₃ min ⁻¹ ml ⁻¹ of oxygen)	78.44±7.57	5.07±0.21	47.00±3.14	320.51±41.90
10 min (0.048 mg O ₃ min ⁻¹ ml ⁻¹ of oxygen)	6.62±4.28	1.41±0.28	4.66±0.01	267.95±6.98

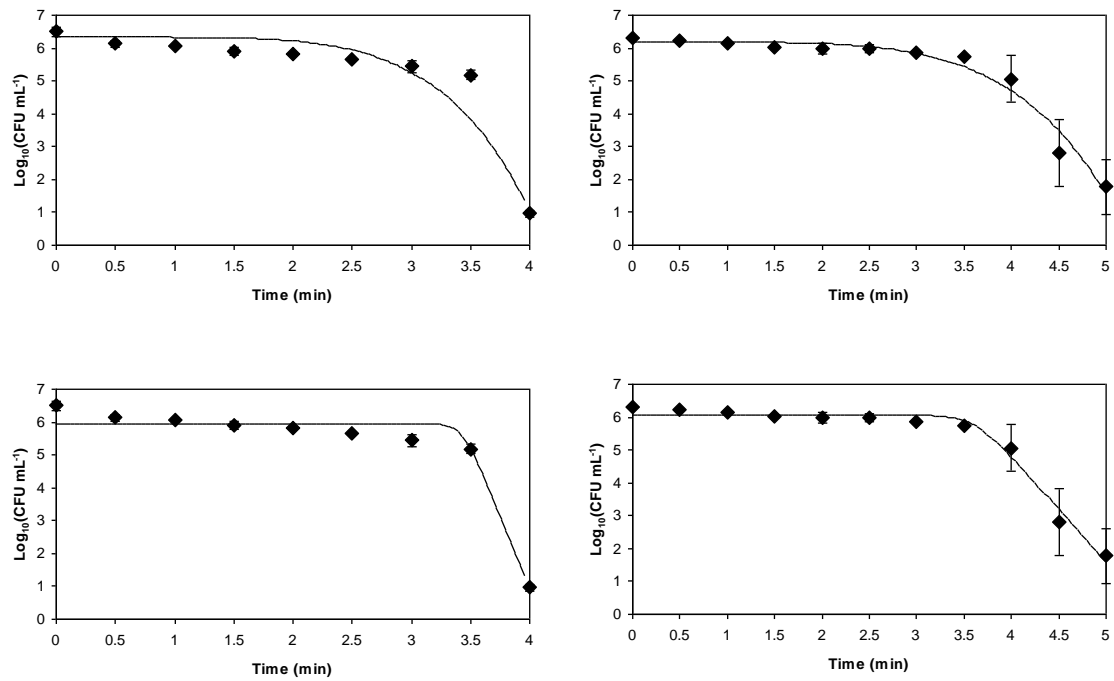


Figure 1: ozone inactivation of non acid exposed cells of *E. coli* Top figure: Weibull model, Bottom figure: Geeraerd and others, (2000) model.

- a) *E. coli* ATCC 25922
- b) *E. coli* NCTC 12900

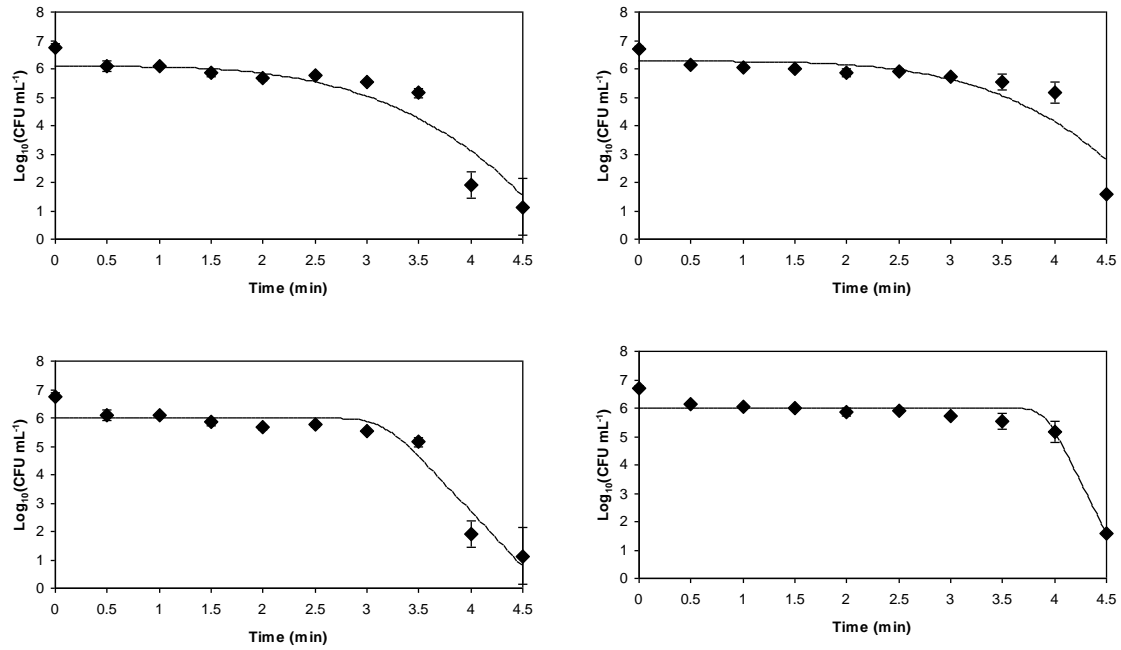


Figure 2: ozone inactivation of 1 h acid exposed *E. coli*. Top figure: Weibull model, Bottom figure: Geeraerd and others, (2000) model.

- a) *E. coli* ATCC 25922
- b) *E. coli* NCTC 12900

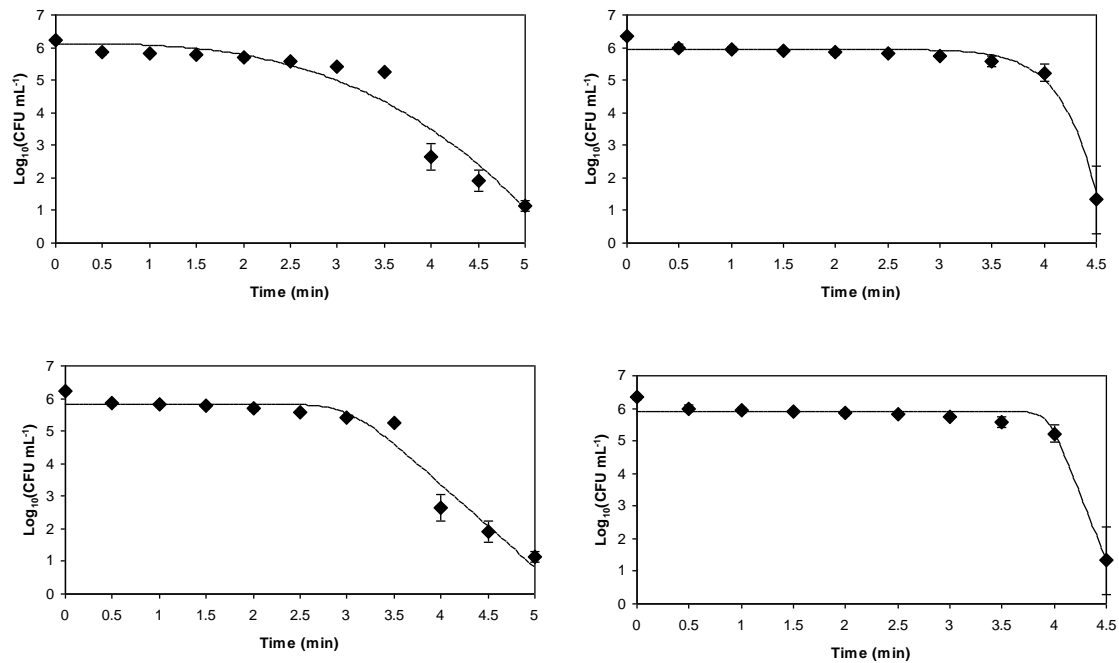


Figure 3: ozone inactivation of 18 h acid exposed *E. coli*. Top figure: Weibull model, Bottom figure: Geeraerd and others, (2000) model.

c) *E. coli* ATCC 25922

d) *E. coli* NCTC 12900

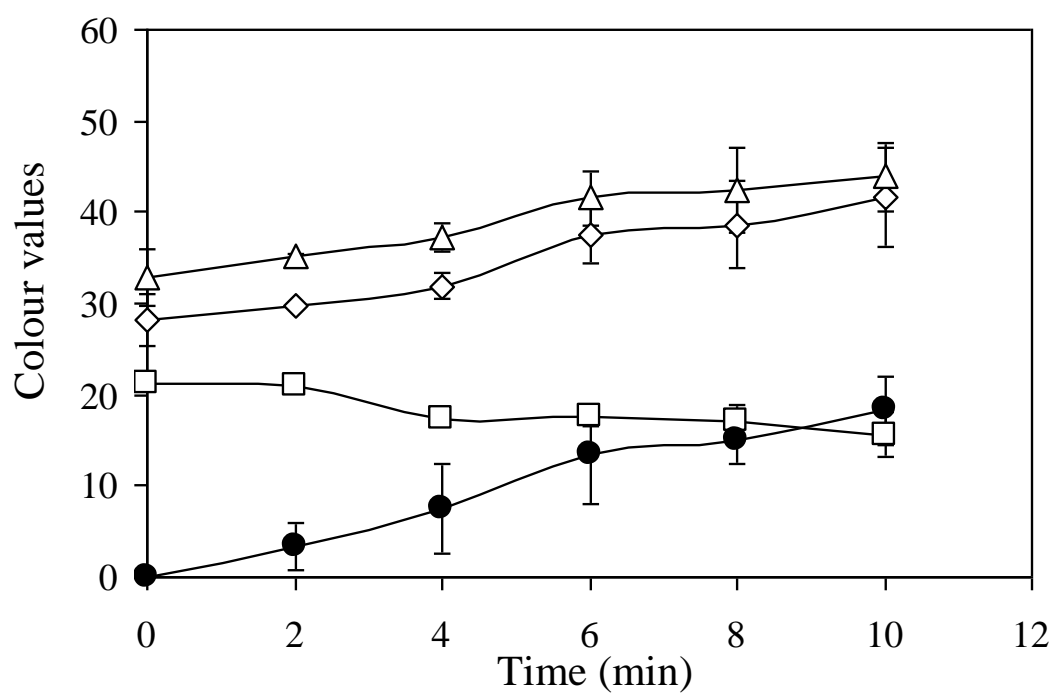


Fig 4. Effect of ozone processing time on the colour values for a) L^* (\diamond) b) a^* (\square), c) b^* (Δ) & TCD (\bullet) values during ozonation at gas flow rate of 0.12 L min^{-1} and ozone concentration of.